FULL NARRATIVE REPORT - TIM WILLSON

RAPIDLY EMERGING ANTIVIRAL DRUG DISCOVERY INITIATIVE (READDI)

Research Activities and Findings

We seek to identify the proteins of host cells that are required for replication of SARS-CoV-2. These host proteins would provide new molecular targets for development of broad spectrum anti-viral drugs for treatment of COVID-19 and future coronavirus pandemics. In the current study, the host kinase CSNK2 was identified as a potential anti-coronavirus drug target through the development of potent small molecule inhibitors.

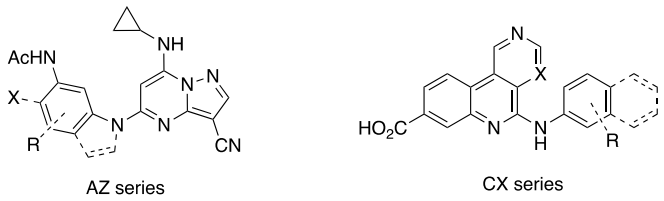


Figure 1 CSNK2 inhibitors

We designed and synthesized analogs in two chemical series (AZ and CX) as potential CSNK2 inhibitors (Figure 1). Using a CSNK2 NanoBRET assay, analogs in the AZ chemical series were identified as potent inhibitors of the kinase in cells with IC50 <10 nM. All of these potent CSNK2 inhibitors were tested for anti-coronavirus activity in DBT cells infected with mouse hepatitis virus (MHV). Multiple analogs in the AZ chemical series were identified that inhibited replication of the MHV coronavirus with IC50 < 100 nM (Figure 2A). The most potent analogs from the AZ series were further tested for anti-viral activity in A549-ACE2 cells infected with SAR2-CoV-2. Three analogs were identified that inhibited SAR2-CoV-2 replication with IC50 < 1 µM (e.g. AZ-I, Figure 2B).

A

Compound	CSNK2 Inhibition IC50 (nM)	MHV Replication IC50 (nM)
UNC-AZ-I	2.9	1.6
UNC-AZ-H	1.2	1.7
UNC-AZ-N	2.2	7.4
UNC-AZ-G	4.1	7.5
UNC-AZ-J	8.9	26
UNC-AZ-T	3.8	47
UNC-AZ-K	14	84

B

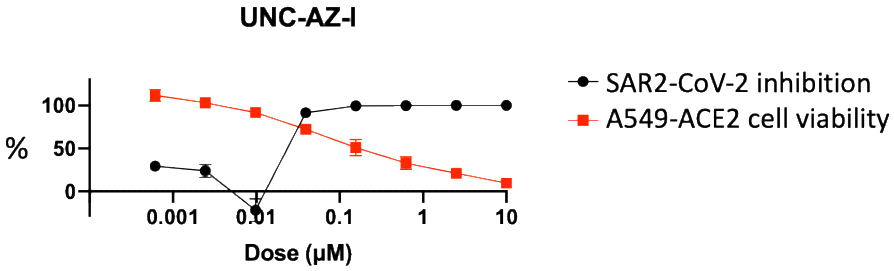


Figure 2 Potent CSNK2 inhibitors in the AZ series inhibit coronavirus replication

In addition, three analogs in the CX series that were identified as potent CSNK2 inhibitors in the NanoBRET assay showed inhibition of the MHV coronavirus replication with IC50 < 3 µM (Figure 3). Compounds from the CX series were less potent at inhibiting coronavirus replication than the best compounds from the AZ series. However, additional optimization of the CX series to improve anti-viral activity has continued as a potential back-up to the lead AZ series.

Compound	CSNK2 Inhibition IC50 (nM)	MHV Replication IC50 (nM)
AA-CS-9-014	0.4	360
AA-CS-9-015	23	2000
AA-CS-9-013	95	2300

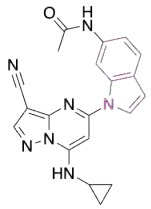
Figure 3 Potent CSNK2 inhibitors in the CX series inhibit coronavirus replication

To support testing in a mouse model of COVID-19, the pharmacokinetic profile of the potent CSNK2 inhibitors from the AZ series was determined. Analogs in the AZ series were found to be rapidly metabolized in mouse liver microsomes. Four analogs with modest stability in liver microsomes were dosed by ip injection in mice, but each of them showed rapid clearance and blood levels too low to support testing in the COVID-19 model (Figure 4A). The indole analog UNC-AZ-T had the highest blood level and was selected as a lead for further optimization to improve its pharmacokinetic properties (Figure 4B). Using computational software to predict metabolic stability, new analogs in the AZ series were designed to resist hepatic metabolism. Synthesis of these analogs of the lead compound UNC-AZ-T is currently in progress in collaboration supported by Takeda (Millennium) Pharmaceuticals.

A

Compound	Liver Microsome @ 30 min (% remaining)	Blood level mice @ 3 h, ip 10mpk (ng/mL)
UNC-AZ-T	59	200
UNC-AZ-G	74	110
UNC-AZ-I	71	62
UNC-AZ-K	70	2
UNC-AZ-O	41	43

B



UNC-AZ-T

✓ High CSNK2 potency
Good selectivity
Good anti-CoV activity

✗ Medium liver microsome stability
High clearance in mice
Low blood levels in mice

Figure 4 Drug properties of anti-viral CSNK2 inhibitors

Budget Overview

			NON-PERSONNEL EXPENSES							
ORIGINAL BUDGET	**REVISED BUDGET**	**PERSONNEL EXPENSES** (Payroll and benefits cost for employee that are dedicated to COVID-19)	**Contracted Labor Expenses**	**Other Service Expenses** (e.g. utilities, telephone, data, lease related expenses)	**Subcontract Expenses** (e.g. construction, maintenance)	**Goods Expenses (e.g. supplies, PPE)**	**Equipment Expenses**	**TOTAL NON-PERSONNEL EXPENSES**	**TOTAL EXPENDITURES**	**BALANCE**
$215,115	**$215,115**	**$92,789.42**	$13,920	$36,527.13	0	$67,870.65	0	**$118,317.78**	**$211,107.20**	**4007.8**

Personnel supported by this award

Personnel	Title	Affiliation
Tim Willson	PI, Research Professor	Structural Genomics Consortium-UNC
Xuan Yang	Postdoctoral Research Associate	Structural Genomics Consortium-UNC

NC Policy Collaboratory project-related Grant (awarded)

Funded collaboration with Takeda (Millenium) Pharmaceuticals that was enabled by the NC Policy Collaboratory grant
Award Title: *Identification of kinase inhibitors as therapies for SARS-CoV-2 and future pandemic viruses*
Award (Ramses Project #): A20-1447
Amount: $444,929